

Shiraz E-Medical Journal

Vol. 14, No. 2, April 2013

<http://semj.sums.ac.ir/vol14/apr2013/91032.htm>

Association of transforming growth factor- β 1 gene polymorphism with Visceral leishmaniasis in an Iranian population

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Received for Publication: November 26, 2012, Accepted for Publication: July 21, 2013.

Abstract

Introduction: Visceral leishmaniasis (VL) or Kala-azar is a parasitic disease caused by a protozoan of Leishmania genus and in Iran by Leishmania infantum. Cytokines have a major role in determining progression and severity of clinical manifestations in VL. The role of TGF- β 1 in VL has been recognized since 1992. We investigated polymorphism in the TGF- β 1 gene, which is a cytokine known to have a role in onset and severity of the disease.

Methods: This descriptive and cross-sectional study was done on 85 patients with confirmed VL, 106 healthy seronegative controls and 99 seropositive controls. Salting out method was used to extract DNA and the amplification refractory mutation system (AR123MS)-PCR procedure was used for detecting polymorphism at TGF β 1 (-509) C/T.

Results: The frequency of TGF- β 1 CC, CT and TT genotypes among all subjects were 21.4%, 71% and 7.6% respectively. Statistical analysis of distribution of genotypes was performed using Chi-Square test and reveal a significant difference among groups (P = 0.00).

Conclusion: To our knowledge this study demonstrates for the first time the association of the cytokine transforming growth factor- β 1 gene polymorphism in Iranian patients with VL. Individuals with the transforming growth factor- β 1 (-509) CT genotype may have increased susceptibility to Visceral leishmaniasis.

Keywords: Transforming growth factor- β 1; Polymorphism; Visceral Leishmaniasis

Introduction

Visceral leishmaniasis (VL) or Kala-azar is a chronic disease caused by protozoan parasites of the *Leishmania donovani* complex: *L. donovani* and *L. infantum/chagasi* that are transmitted by a female sandfly (1-3). The disease is characterized by suppression of cell-mediated immune responses and unchecked parasite replication in macrophages of the liver, spleen, and bone marrow (2, 4). VL is predominantly pediatric with a large spectrum of clinical manifestations ranging from asymptomatic to a severe visceral involvement. Clinical disease is caused by parasites infecting the internal organs. In addition to effects due to inherent characteristics of the infecting parasite, the immune response against *Leishmania* can be substantially modified by factors such as the nutritional status, age, and genetic background of the host (1). Most human infections caused by the visceralizing strains of *Leishmania* run a subclinical and self-healing course, but in some a fatal visceral disease evolves for which treatment is required (2).

Transforming growth factor-beta (TGF- β) is a 25kDa disulphide-linked homodimer or heterodimer protein with a broad range of

biological functions (5). This multifunctional cytokine is produced by, and affects, every cell type in the human body. The gene encoding TGF- β 1 has been mapped to the long arm of chromosome 19 (4, 6). In mammalian cells, there are three subtypes of TGF- β (TGF- β 1, TGF- β 2, and TGF- β 3). TGF- β 1 is the most abundant form. This molecule regulates cellular processes by binding to the receptor type II (TGF- β RII), resulting in binding and phosphorylation of the receptor type I (TGF- β RI). TGF- β RI then phosphorylates and activates SMADs. The SMADs complex then translocates into the nucleus and interacts with DNA directly or indirectly through other DNA-binding proteins, resulting in the transcription of the target genes and thus leading to proliferation and differentiation of cells, the release of cytokines and angiogenesis (7).

The role of TGF- β 1 in Kala-azar has been recognized since 1992. TGF- β 1 is directly involved in monocyte/macrophage activation, inhibiting the production of nitric oxide and oxygen free radicals that exert microbicidal activity against the parasite. It also inhibits production of the pro-inflammatory cytokine IFN- γ by lymphocytes, directing the immune response

to a Th2 profile and contributing to progression to a more severe form of VL in the infected patient. Studying peripheral blood mononuclear cells of patients with Kala-azar and post Kala-azar, from two endemic regions in India, showed increased production of TGF- β 1 in response to specific antigen in patients with the active form of the disease (1). TGF- β 1 plays an important role in the progression of leishmaniasis in rodents. TGF- β 1 enhances the progression, or prevents the cure of leishmaniasis in murine models (4).

The level of TGF- β 1 is under genetic control. Polymorphisms have recently been described for TGF- β 1.2, 3. These polymorphisms were reported to affect the production of TGF- β proteins (5). Some of these polymorphisms are located in the promoter region at positions such as 988, 1572, 800, and 509. The -800 G/A and -509 C/T single nucleotide polymorphisms (SNPs) of the TGF- β 1 gene were not distributed similarly among different populations or diseases (7, 8). Both of these polymorphisms are functional and could affect the level of TGF- β 1 secretion (7). For example, the plasma concentrations of TGF- β 1 were approximately twice as high in homozygous individuals for the T allele compared with the concentrations for

homozygous individuals for the C allele at position -509 in the TGF- β 1 promoter region (9).

Our objective was to investigate the influence of the TGF- β 1 gene (-509) C/T polymorphisms on the susceptibility to Visceral leishmaniasis in a group of Iranian patients.

Methods and Materials

Patients and controls

In this study, three groups of individuals were recruited from meshkin shahr health centers. The patient group comprised 85 clinically and paraclinically confirmed visceral leishmaniasis. Second group comprised 99 serologically positive for VL by IFA test, but without any signs and symptoms and history of confirmed VL disease as well. Third group comprised also 106 healthy individuals without any history of VL and negative for VL serologically. Demographic data of patients were obtained from their medical records. From each subject, 10 mL of venous blood was collected into tubes containing 50 mM/L EDTA.

DNA extraction and TGF β 1 genotyping

Genomic DNA was isolated by the salting-out method. Briefly, 5mL of blood was mixed with lysis buffer (EDTA 1mmol/L, NaCl 15mmol/L, TriseHCl 15mmol/L, pH

7.5). Leukocytes were spun down and washed with H₂O. The pellet was incubated with proteinase K at 56 °C and subsequently salted out at 4°C using a saturated NaCl solution. Precipitated proteins were removed by centrifugation. The DNA in the supernatant fluid was dissolved in 5mL H₂O. Amplification refractory mutation system–polymerase chain reaction (ARMS-PCR) method was used to genotype C–509T TGF–β1 polymorphism under investigation using primers as already described (10). Briefly, two complementary reactions were established for each allele consisting of target DNA, allele specific ARMS primer (AR1 or AR2) and the common primer (CF). A 349 base pair region in the TGF-β1 promoter was targeted for amplification. The sequences of primers used in the study are AR1 5' AAGGGGCAACAGGACACCTGGG 3', AR2 5' AAGGGGCAACAGGACACCTGGA 3' and CF 5' CTACGGCGTGGAGTGCTGAG 3'. Amplification was carried out using a PCR Techne Flexigene apparatus (Roche, Mannheim, Germany) in a total volume of 15 µl that contained 100 ng of genomic DNA, each primer pair consisting of 2 µmol of allele specific and 0.5 µmol of common primers, 200 µmol L⁻¹ each dNTP; 10 mM

Tris–HCl (pH 8.3); 50 mM KCl, 1.5 mM MgCl₂ and 0.5 IU Taq DNA polymerase. PCR performed without DNA template represented the negative control. Amplification was carried out for 35 cycles, each cycle consisting of denaturation at 94°C for 30 s, annealing at 61°C for 20 s, extension at 72°C for 20 s and finally a 3 min extension at 72°C. The amplified PCR products were analysed by 2% agarose gel electrophoresis followed by 0.5 µg mL⁻¹ ethidium bromide staining and ultraviolet visualization.

Statistical analysis

SPSS version 19 software (SPSS, Chicago, IL), Chi square test were used to study the differences in genotype and allele frequencies between patients and controls. P < 0.05 was considered statistically significant.

Results

Table 1 summarizes the genotype distribution and allele frequencies of TGF-β1 gene polymorphisms at -509 C/T in healthy controls and patients with visceral leishmaniasis.

Of the 106 healthy control subjects, 5 had the CC genotype, 75 the CT type and 5 the TT type. Of the 99 serologically positive for VL subjects, 27 were CC type, 66 were CT and 6 were TT. Of the 85 patients with

visceral leishmaniasis, 30 were CC type, 65 were CT and 11 were TT. The frequency of TGF- β 1 CC, CT and TT genotypes among all subjects were 21.4%, 71% and 7.6% respectively. According to the results, -509 C/T was the dominant genotype among the groups.

Statistical analysis of distribution of genotypes was performed using Chi-Square test and reveal a significant difference among groups ($P = 0.00$).

Our study provides evidence for an association between TGF- β 1 -509 C/T polymorphism and susceptibility to visceral leishmaniasis.

Discussion

To our knowledge this study demonstrates for the first time the association of the cytokine transforming growth factor- β 1 gene polymorphism in Iranian patients with VL. We found that Individuals with the transforming growth factor- β 1 (-509) C/T genotype may have increased susceptibility to VL.

TGF- β 1 is an immunoregulatory and predominantly an immunosuppressive cytokine. It has been suggested that the -509 (C/T) polymorphism is significantly associated with the plasma concentration of TGF- β 1. Some studies indicated that polymorphisms in the promoter region of

this cytokine resulted in altered transcriptional regulation and thereby, might influence the development and severity of TGF- β 1-related diseases. The -800 (G/A) substitution is thought to disrupt a consensus half-site for binding of the nuclear transcription factor CRE binding protein, that consequently leads to a lower production of total TGF- β 1 in the circulation. On the other hand, the T allele of the -509 (C/T) polymorphism has been reported to be associated with a higher transcriptional activity and therefore, higher production of total and active TGF- β 1 (11).

Associations between genetic polymorphisms in TGF- β 1 gene and disease status have been described in a diverse range of diseases, particularly with non-infectious diseases such as asthma, cancer, arthritis, osteoporosis, myocardial infarction and systemic lupus erythrmatosus. Only a few studies have evaluated the influence of these gene variants on infections, namely brucellosis and tuberculosis. A significant association has only been found between SNP at codons 10 and 25 of TGF β 1 and the risk of brucellosis (12).

Our results is in agreement with a previous report on the -509 C/T polymorphism in a group of Brazilian patients with Visceral leishmaniasis. In that report, the presence of

the T variant in position -509 of the TGF- β 1 gene, was significantly increased in VL and DTH + (positive delayed-type hypersensitivity) individuals compared to DTH- (negative delayed-type hypersensitivity) controls (1).

Jose E. Calzada and coworkers studied several single-nucleotide polymorphisms (SNP) in the TGF- β 1 gene in a Peruvian and Colombian population of patients with Chagas disease and healthy controls. They found that the presence of the T allele or T/T genotype coding for low production of TGF- β 1 was found to be consistently higher in healthy individuals than in patients with Chagas disease, suggesting a possible protective effect of this allele to *T. cruzi* infection. Conversely, the frequency of the high producer allele C and the genotype C/C at codon 10 was invariably increased in the patients groups, indicating that this allele may be a risk factor for genetic susceptibility to Chagas disease. In the other hand, there was no difference in the genotype and allele distribution of -509 C/T and 10 T/C TGF- β 1 genetic variants among asymptomatic and cardiac individuals, indicating no influence of these polymorphisms on Chagas disease progression (12). Fan XY and coworkers studied the association between genetic

polymorphisms of TGF- β 1 and susceptibility to pneumoconiosis. They conclude that TGF- β 1 (-509) CC genotype may be the protective factor for the pneumoconiosis. The control group that was workers exposed to dust but without pneumoconiosis carrying TGF- β 1 (-509)T allele were more susceptible to pneumoconiosis (13).

Our study has some limitations. We have analyzed one variants within the TGF- β 1 gene that were frequently found in the studied populations. Other polymorphisms with known influence on differential TGF- β 1 expression have been reported and thus, their analyzes will strength our results. Another limitation is that this study was restricted to only one Visceral leishmaniasis disease endemic populations in Iran. Our results should be validated in other endemic populations with different genetic background and with different eco-epidemiological features regarding Visceral leishmaniasis disease.

Conclusion

Our findings suggest an association between TGF- β 1 polymorphism and susceptibility to Visceral leishmaniasis in Iranian patients. Individuals with the transforming growth factor- β 1 (-509) CT genotype may have

increased susceptibility to Visceral leishmaniasis.

Ethical issues

No ethical issues to be declared.

Conflict of interests

No conflict of interest to be declared.

Acknowledgements

This research has been supported by Young Researchers Club, Hamedan Branch, Islamic Azad University, Hamedan, Iran. We acknowledge Dr S. Sobhan for his collaboration.

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